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# Effect of Bone Marrow-Derived Stem Cells on the Submandibular Salivary Glands of Streptozotocin-induced Diabetic Rats

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## Abstract

***Background*: Stem cells are one of the most fascinating areas of biology today they play an important role in our bodies from embryonic development through adulthood, and diabetes mellitus (DM) is one of the most common degenerative diseases that affect all body tissues and organs. *Objectives*: The aim of this study was to evaluate the effect of bone marrow derived stem cells on the submandibular salivary glands in diabetic rats. *Materials and methods* A Total of sixty, adult male albino rats with body weight ranging from 180-200 grams were used in the present investigation. They were divided as follows, Group 1 (15 animals) served as controls. Group 2 (15 animals), they were subjected to a single intraperitoneal injection of streptozotocin. Group 3: consisted of 30 animals, they were subdivided as follows: Subgroup 3.1 (15 animals), they were used for isolation and culture of bone marrow stem cells and Subgroup 3.2: (15 animals) they were subjected to a single intraperitoneal injection of streptozotocin, the same as group 2, in a dose of 60 mg/kg body weight for induction of diabetes mellitus. One week later, they were subjected to a single intravenous infusion of mesenchymal bone marrow stem cells. At the end of the experiment the animals of the different groups were sacrificed by cervical dislocation, their submandibular salivary glands dissected out, separated into right and left glands for histological and ultrastructural examination. *Results* Light and transmission electron microscopic results showed the degenerative changes of diabetes in group 2 and the high regenerative changes of bone marrow derived stem cells in group 3.2 compared to the control group 1. *Conclusion* Stem cells have magnificent regenerative effect in diseased and injured tissues in different organs.**

***Keywords:*** Parenchymal elements, embryonic development, adulthood, transmission electron microscope.

## Introduction

Diabetes mellitus represents a group of diseases of heterogeneous etiology, characterized by chronic hyperglycemia and other abnormalities in carbohydrate, lipid and protein metabolism which are caused by deficiency of insulin effect[(1)]. Concerning the oral complications of diabetes mellitus, they may include, but are not necessarily limited to: gingivitis and periodontal disease, xerostomia and salivary gland dysfunction with increased susceptibility to bacterial, viral and fungal infections den-

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tal caries, periapical abscesses, loss of teeth, impaired ability to wear dental prosthesis, taste impairment, lichen planus and burning mouth syndrome[2] which is difficult to be treated with normal medications. Stem cells are biologically undifferentiated cells in all multicellular organisms with various degrees of potency, plasticity and they are capable of proliferation, self-renewal, conversion to differentiated cells and regenerating tissues[3]. So, it can be used to overcome the complications of diabetes related to salivary gland dysfunction.

Stem cells have been divided according to their potency into: *Totipotent cells* which have the ability to divide and produce all the differentiated cells in an organism including extra embryonic tissues[4]. *Pluripotent cells*, they are the descendants of the totipotent cell, they have potential to differentiate into any kind of the three germ layers endoderm, mesoderm and ectoderm[5]. *Oligopotent cells*, these are stem cells that divide into few types of cells as lymphoid or myeloid stem cells[6]. *Unipotent cells,* these are stem cells that have limited potential to generate only one specific cell type of their own tissue of origin. [7]. According to their sources, stem cells have been divided into two categories, Embryonic stem cells and Somatic stem cells, also called adult stem cells or mesenchymal stem cells[8].

## Materials and Methods

A total of sixty, adult male albino rats with body weight ranging from 180-200 grams were used in the present investigation. They were divided as follows: *Group 1 (control group):* consisted of 15 animals and served as controls. *Group 2 (diabetic group):* consisted of 15 animals, they were subjected to a single intraperitoneal injection of streptozotocin in a dose of 60 mg/kg body weight dissolved in 0.1 M. citrate buffer PH 4.5 for induction of diabetes mellitus. *Group 3:* consisted of 30 animals, they were subdivided as follows: *Subgroup 3.1 (donor group)*: consisted of 15 animals, they were used for isolation and culture of bone marrow stem cells. *Subgroup 3.2 (diabetic group treated with stem cells):* consisted of 15 animals, they were subjected to a single intraperitoneal injection of streptozotocin, the same as group 2, in a dose of 60 mg/kg body weight for induction of diabetes mellitus. One week later, group 3.2 were subjected to a single intravenous infusion of mesenchymal bone marrow stem cells (MBMSCs) to study the effect on the submandibular salivary glands of diabetic rats. The cell number were calculated by hemocytometer for intravenous administration [$(1.5x10^3)^{-10^6}$] cells per rat in 0.2 ml phosphate buffer saline and slowly injected into the tail vein of anaesthetized rat over two minutes period using a 22 gauge needle[9]**.** That is to study the effect of the injected mesenchymal bone marrow stem cells on the submandibular salivary glands of diabetic rats. Verifying induced diabetes carried out by detecting hyperglycemia (200mg/dl or more) in a sample of blood collected in heparinized capillary tubes from the retro-orbital plexus of veins from 12 hours fasting animals.

*Preparation of mesenchymal bone marrow derived stem cells*[10]**:** Bone marrow will be harvested by flushing the tibiae and femurs of the six weeks old rats with Dulbecco's modified Eagle's medium (DMEM, Gibco/ BRL) supplemented with 10% fetal bovine serum (Gibo/BRL). Nucleated cells will be isolated with a density gradient [Fico 11/paque (Pharmacial)] and will be resuspended in complete culture medium supplemented with 1% penicillin- streptomycin (Gibo/BRL). Cells will be incubated at 37˚C in 5% humidified $CO_2$ for 12-14 days as primary culture or upon formation of

larger colonies. When large colonies develop (80-90% confluence), cultures will be washed twice with phosphate buffer saline and the cells will be tripsinized with 0.25% trypsin in 1mm EDTA for 5 minutes at 37˚C. After centrifugation, cells will be resuspended with phosphate buffer saline. The mesenchymal stem cells (MSCs) in culture will be characterized by their adhesiveness and fusiform shape and by detection of CD29 (one of the surface markers of MSCs, by RT-PCR). The animals of all groups will be fed natural diet and supplied drinking water throughout the whole experimental period which lasted for two months. At the end of the experiment which last for two months after the induction of diabetes and injection of stem cells in group 2 and 3.2 respectively, the animals of the different groups were sacrificed by cervical dislocation, their submandibular salivary glands dissected out, separated into right and left glands. Those of the right side were fixed in 10% neutral buffered formalin, processed and embedded in paraffin. Six microns thick sections were cut to be stained with hematoxylin and eosin for histological examination to detect any structural changes in the paranchymal and stromal elements of salivary glands. Specimens of the left side were used for transmission electron microscopic examination. Small pieces of average size 1 cubic mm were immediately cut, rapidly fixed in 3% phosphate buffered gluteraldehyde, post fixed in 1% buffered osmium tetroxide, then embedded in epoxy resin. Ultrathin sections were cut to be stained with uranyl acetate and lead citrate to be examined using TEM (Joel 1000) in the Cancer Institute Cairo University.

## Results

*I. Histological Results using Hematoxylin and eosin stained sections*

*1. Group 1 animals (control group):* the histological examination of the control group revealed the normal histology of the submandibular salivary glands, being composed of acinar portions and duct system within normal connective tissue (CT) stroma (Fig. 1).

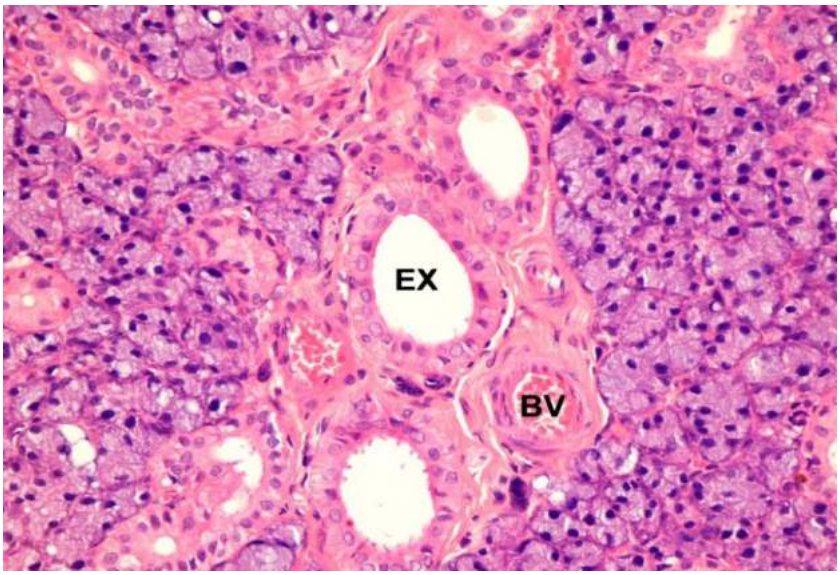


**Fig. 1:** A photomicrograph of the submandibular salivary gland of a control rat showing excretory ducts (EX) in connective tissue strand with many blood vessels (BV). *(Hx&E. orig. mag. 200)*

2. *Group 2 animals (diabetic group):* The most outstanding and common finding was the apparent reduction in the size and number of the secretory terminal portions and ducts of the submandibular salivary gland with marked spacing between acini. The acinar cells showed numerous intracytoplasmic vacuolizations and reduced basophilia of their cytoplasm. The GCTs revealed decreased eosinophilic granular content and areas of degeneration could be seen in their lining cells (Fig. 2).

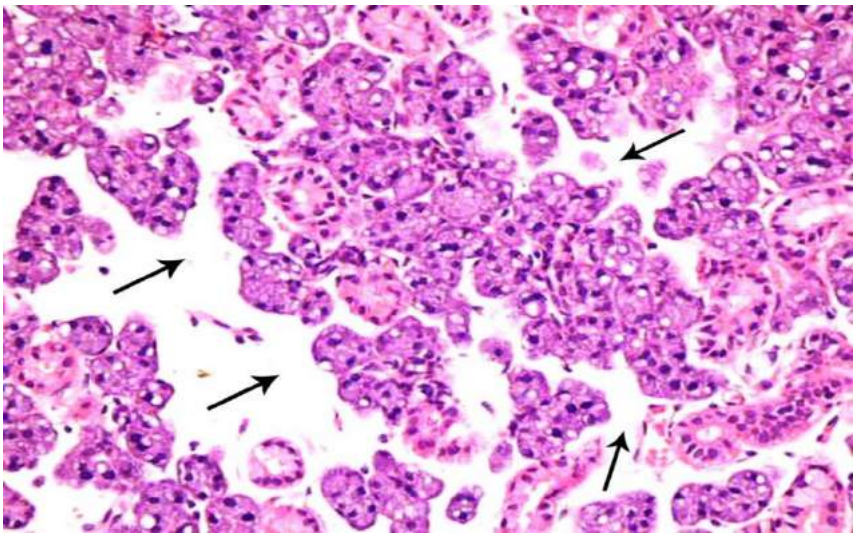

**Fig. 2:** A photomicrograph of the submandibular salivary gland of a diabetic rat showing spacing between groups of serous acini (arrows). *(Hx&E. orig. mag. 200)*

The striated duct cells revealed numerous intracytoplasmic vacuolizations and shrinkage of their nuclei. excretory ducts revealed thinning of their epithelial lining with stagnated secretion in their wide lumen. The C.T stroma showed increased fibrous content with dilated blood vessels engorged with RBCs and inflammatory cells infiltration (Fig. 3).

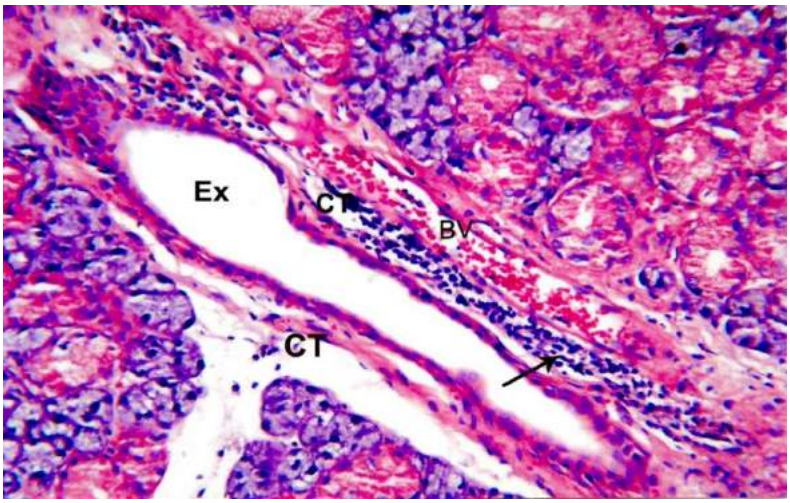


**Fig. 3:** A photomicrograph of the submandibular salivary gland of a diabetic rat showing excretory duct with flattened cell lining (EX) surrounded with connective tissue (CT) septa with degenerated collagen fibers, dilated blood vessels (BV) and inflammatory cell infiltrate (arrow). *(Hx&E. orig. mag. 200)*

*3. Subgroup 3.2 (diabetic animals injected with mesenchymal bone marrow derived stem cells (MBMDSCs):* There was marked improvement in the structural elements of the submandibular salivary gland. There was total or subtotal absence of the intracytoplasmic vacuolizations both in the secretory terminal portions and ducts of the diabetic rats. Granular convoluted tubules regained their eosinophilic and granular content of their cytoplasm and the ducts regained their normal lining and lumena with no retained secretion (Fig. 4). The connective tissue stroma appeared normal with absence of inflammatory cells and normally appearing blood vessels (Fig. 5).

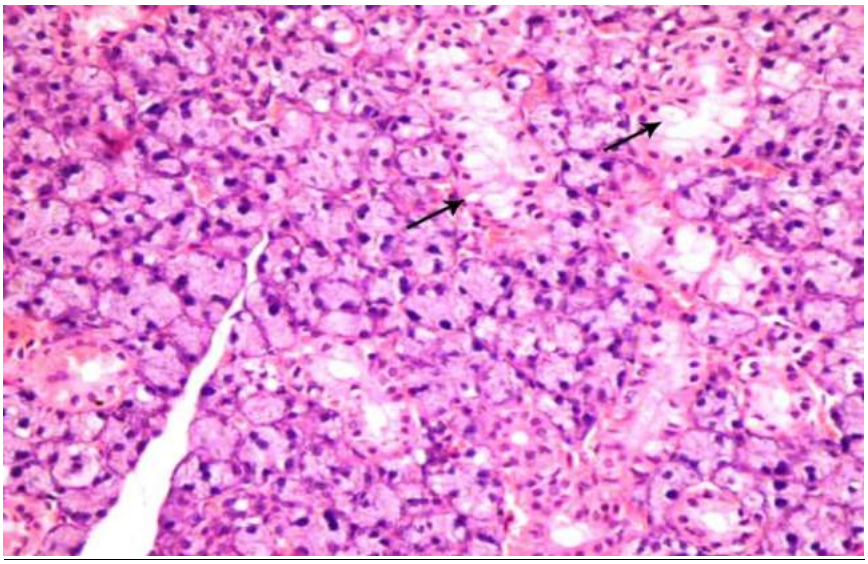

**Fig. 4:** A photomicrograph of the submandibular salivary gland of a diabetic rat treated with mesenchymal bone marrow derived stem cells showing minimal intracytoplasmic vacuolizations and swelling of mucous acinar cells (arrows). *(Hx&E. orig. mag. 200)*

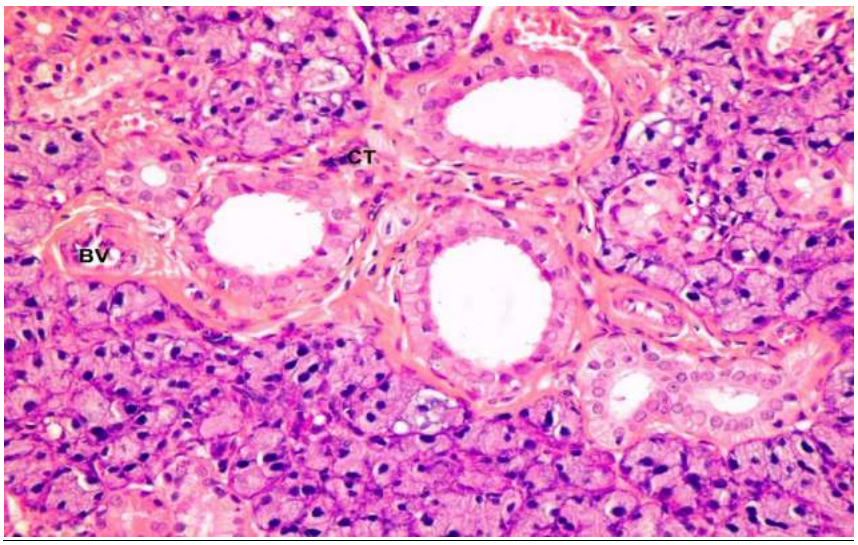


**Fig. 5:** A photomicrograph of the submandibular salivary gland of a diabetic rat treated with bone marrow derived stem cells showing excretory ducts in fibrous connective tissue (CT) septa with normal appearing blood vessels (BV). *(Hx&E. orig. mag. 200)*

*II. Ultrastructural results using Transmission electron microscope (TEM):*

*1. Group 1 animals (control group):* The submandibular salivary gland of the control group revealed normal ultrastructural findings with normal acinar and duct cells appearance, normal cell organelles and desmosomal junctions and junctional complexes were frequently seen between cells. The connective tissue stroma was formed of moderately dense collagen fibers, fibroblasts, rich with their different types of organelles mainly rough endoplasmic reticulum (RER), mitochondria and Golgi apparatus together with an oval euchromatic nuclei (Fig. 6).

*2. Group 2 animals (diabetic group):* Electron microscopic examination of the submandibular salivary glands of diabetic rats revealed marked degenerative changes in the ultrastructure of its parenchymal and stromal elements. There were marked in tractoplasmic vacuolization in the acinar, duct and granular convulated tubules (GCTs) cells with pyknotic nuclei with irregular nuclear membranes and mostly heterochromatin. The cells revealed degenerated cell organelles. Marked decrease in the granular content of (GCTs), and loss of basal infolding of striated duct cells were consistent findings. The connective tissue stroma of the gland showed signs of degeneration and dissociation of the collagen fibers, fibroblasts and markedly dilated blood vessels engorged with blood (Fig. 7).

*3. Subgroup 3.2 animals (diabetic injected with mesenchymal bone marrow derived stem cells):* They revealed marked regeneration of the parenchymal and connective tissue (CT) elements of the glands with more or less normal findings resembling those of the control group with absence of the degenerative effects changes that were seen in the diabetic group (Fig. 8).

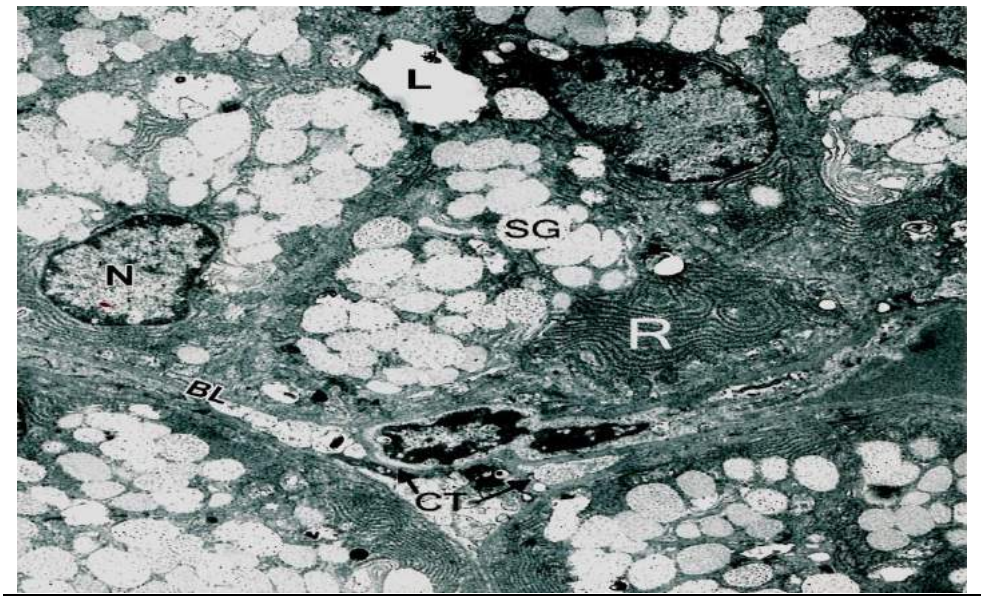


**Fig. 6:** An electronmicrograph of the submandibular salivary gland of a control rat showing serous acinus with pyramidal cells surrounding narrow lumen (L). Each cell had rounded nucleus (N), rough endoplasmic reticulum (R) and secretory granules (SG). The basal plasma membrane of the cell rest on basal lamina (BL) that joined it to the outside connective tissue (CT). *(Uranyl acetate & lead citrate X1700)*

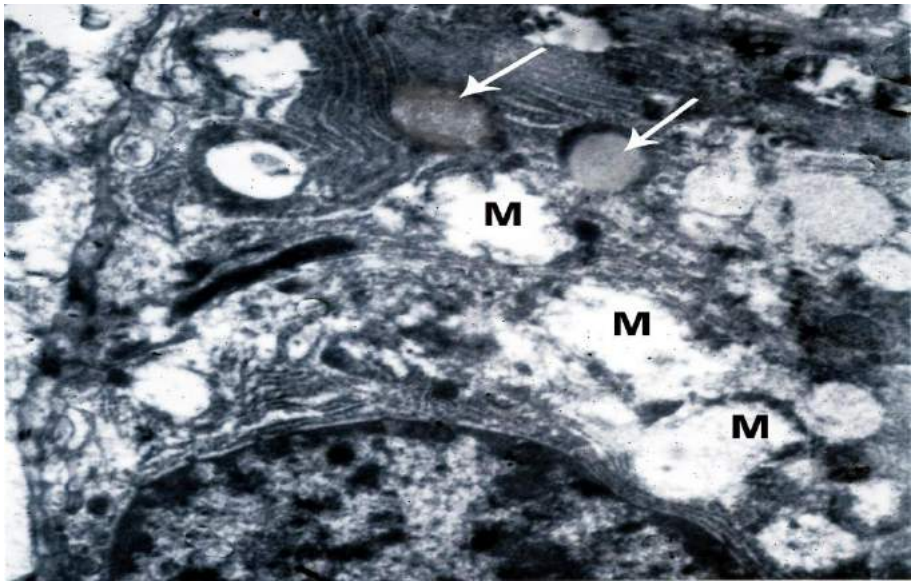


**Fig. 7:** An electronmicrograph of the submandibular salivary gland of a diabetic rat showing serous acinar cell with fat droplets (arrow) and degenerated mitochondria (M). *(Uranyl acetate & lead citrate X3000)*

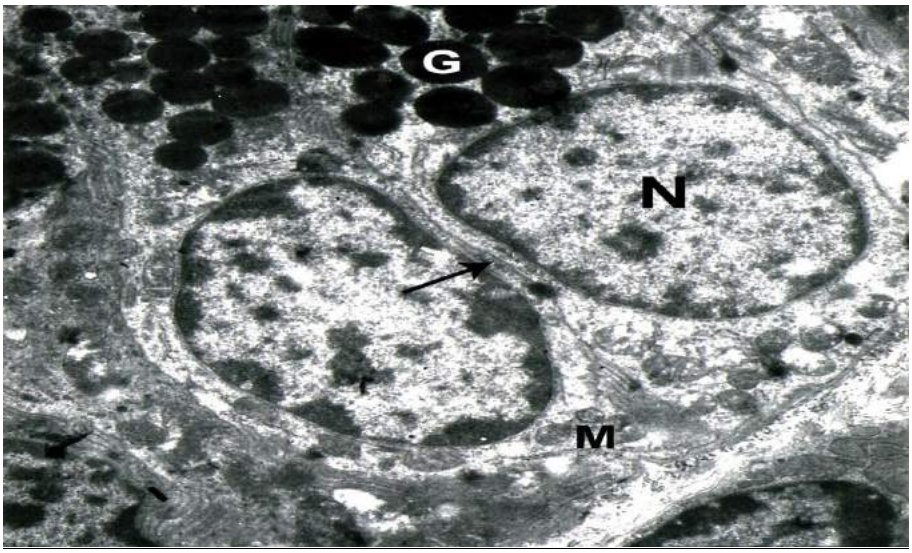


**Fig. 8:** An electronmicrograph of the *submandibular* salivary gland of a rat from group 3.2 showing cells of granular convoluted tubule (GCT) with electron dense granules (G), normal nuclei (N), mitochondria (M) and desmosomal junctions (arrow). *(Uranyl acetate & lead citrate X2000)*

## Discussion

In the present study, there was a deformative changes in the shape of acinar, granular convoluted tubules (GCTs), striated and excretory ducts cells of submandibular salivary glands of diabetic rats. This could be related to disturbance of the contractile proteins in myoepithelial cells of acini and as well as in duct cells. These results were parallel to Drenckhahn et al., who stated that actin and myosin were localized in various major salivary major salivary glands myoepithelial cells[11]. The intracytoplasmic vacuolizations noticed in the current study were consistent with the large vacuoles reported in the cytoplasm of many of the acinar cells in STZ-diabetic rat submandibular

glands, 14 days after STZ treatment. [12] The authors added that these vacuoles appeared to be of a lipid nature since in some sections the material was osmophilic. However, most of the lipid seems to have been removed during fixation and processing of the tissue. The overall appearance of many of the cells was indicative of the advanced stages of degeneration often leading to cell death[12]. The results of the effect of stem cells on salivary glands go with a previous study used stem cell transplantation to functionally reduce salivary gland deficiency when exposed to radiation. The salivary gland treatment resulted in long-term restoration of salivary gland morphology and function[13]. It has been confirmed that the stem cell could restore the gland functionality when transplanted into mice with radiation induced glandular dysfunction. The stem cells were able to self-renew and regenerate the radiation damaged salivary gland[14]. Many research works have proved the regenerative effects of stem cells which made the stem cells a rich source for therapeutic uses in many disorders as in cardiovascular system disorders where the stem cells showed outstanding results in regenerating heart tissues[15], in metabolic disorders[16], in neurological disorders and in many other tissues[17]. This mean that the intake of the bone marrow derived mesenchymal stem cells (BMDMSC) had a great impact on changing the tissue histology.

## Conclusions

*From the present investigation, the following could be concluded:* Diabetes mellitus has damaging effects on the salivary glands of albino rats. The most obvious effects of diabetes on the submandibular gland were lipid accumulation within the acinar and duct cells accompanied with reduced cytoplasmic basophilia, cytoplasmic vacuoliation and mitochondrial degeneration. The granular convoluted tubules (GCTs) were noticeably affected as revealed by the reduction in their apical eosinophilic granular content. Stem cells are undifferentiated cells that undergo self renewal, proliferation and differentiation into specialized cell types. Bone marrow-derived stem cells can be used in regenerating tissues that were damaged due to diabetes. Stem cells can be considered and used as powerful tool to treat wide variety of disorders and diseases that need regeneration of destroyed or damaged tissues.

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